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PATENT

METHOD OF REGULATING BIOLOGICAL ACTIVITY OF PITUITARY TUMOR
TRANSFORMING GENE (PTTG)1 USING PTTG2

The U.S. Government has a paid-up license in this invention and the right in limited
5 circumstances to require the patent owner to license others on reasonable terms as provided for
by the terms of Contract CA75979, awarded by the National Cancer Institute of the National
Institutes of Health.

This application is a continuation-in-part of U.S. Serial No. 09/777,422, filed February
5, 2001, which is a continuation-in-part of U.S. Serial No. 09/730,469, filed December 4, 2000,
10 which is a continuation-in-part of U.S. Serial No. 09/687,911, filed on October 13, 2000, which
is a continuation-in-part of U.S. Serial No. 09/569,956, filed on May 12, 2000, which is a
continuation-in-part of U.S. Serial No. 08/894,251, filed on July 23, 1999, as a national stage
application, under 35 U.S.C. § 371, of international application PCT/US97/21463, filed
November 21, 1997, which claims the priority of the filing date of U.S. Provisional Application
15 Serial No. 60/031,338, filed November 21, 1996.

BACKGROUND OF THE INVENTION

Throughout the application various publications are referenced in parentheses. The
disclosures of these publications in their entireties are hereby incorporated by reference in the
application in order to more fully describe the state of the art to which this invention pertains.

20 1. Field of the Invention

The present invention relates to a method of inhibiting neoplastic cellular proliferation
and/or transformation of mammalian cells, in vitro and in vivo.

2. Related Art

Pituitary Tumor Transforming Gene (PTTG) is highly expressed in pituitary tumors and
25 neoplasms from the hematopoietic system and colon. (Zhang, X. *et al.*, *Structure, expression,
and function of human pituitary tumor-transforming gene (PTTG)*, Mol. Endocrinol. 13:156-66
[1999a]; Zhang, X. *et al.*, *Pituitary tumor transforming gene (PTTG) expression in pituitary*

- adenomas, *J. Clin. Endocrinol. Metab.* 84:761-67 [1999b]; Heaney, A.P. *et al.*, *Pituitary tumor transforming gene in colorectal tumors*, *Lancet* 355:712-15[2000]; Dominguez, A. *et al.*, *hpttg, a human homologue of rat pttg, is overexpressed in hematopoietic neoplasms. Evidence for a transcriptional activation function of hPTTG*, *Oncogene* 17:2187-93 [1998]; Saez, C. *et al.*, *hpttg is over-expressed in pituitary adenomas and other primary epithelial neoplasias*, *Oncogene* 18:5473-6 [1999]). *PTTG1* is expressed at low levels in most normal human tissues. (Chen, L. *et al.*, *Identification of the human pituitary tumor transforming gene (hPTTG) family: molecular structure, expression, and chromosomal localization*, *Gene*. 248:41-50 [2000]; Heaney, A.P. *et al.* [2000]).
- 10 Levels of *PTTG* expression positively correlate with pituitary and colorectal tumor invasiveness (Zhang, X. *et al.* [1999b]; Heaney, A.P. *et al.* [2000]) and are induced by estrogen. (Heaney, A.P. *et al.*, *Early involvement of estrogen-induced pituitary tumor transforming gene and fibroblast growth factor expression in prolactinoma pathogenesis*, *Nat. Med.* 5:1317-21 [1999]). In tumor cells, *PTTG* mRNA and protein expressions are cell cycle-dependent and peak
- 15 at G2/M phase. (Yu, R. *et al.*, *Pituitary Tumor Transforming Gene (PTTG) Regulates Placental JEG-3 Cell Division and Survival: Evidence from Live Cell Imaging*, *Mol. Endocrinol.* 14:1137-1146 [2000]). The mechanism of *PTTG* action is not very clear. *PTTG* upregulates basic fibroblast growth factor secretion (Zhang, X. *et al.* [1999a]), and transactivates DNA transcription (Dominguez, A. *et al.* [1998]; Wang, Z. *et al.*, *Pituitary tumor transforming gene*
- 20 (*PTTG*) *transactivating and transforming activity*, *J. Biol. Chem.* 275:7459-61[2000]).
- PTTG* encodes a securin protein the expression of which causes cell transformation, induces the production of basic fibroblast growth factor (bFGF), is regulated in vitro and in vivo by estrogen, and inhibits chromatid separation. (Pei, L., and Melmed S., *Isolation and characterization of a pituitary tumor transforming gene*, *Mol. Endocrinol.* 11:433-441 [1997];
- 25 Zhang, X., *et al.*, *Structure, expression, and function of human pituitary tumor-transforming gene (PTTG)*, *Mol. Endocrinol.* 13:156-166 [1999a]; Heaney, A.P., *et al.*, *Early involvement of estrogen-induced pituitary tumor transforming gene and fibroblast growth factor expression in prolactinoma pathogenesis*, *Nature Med.* 5:1317-1321 [1999]; Zou, H., *et al.*, *Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis*,
- 30 *Science* 285:418-422 [1999]).

By dysregulating chromatid separation, *PTTG* overexpression also leads to aneuploidy, i.e., cells having one or a few chromosomes above or below the normal chromosome number. (Zou *et al.* [1999]; Yu, R. *et al.* [2000]). At the end of metaphase, securin is degraded by an anaphase-promoting complex, releasing tonic inhibition of separin, which in turn mediates degradation of cohesins, the proteins that hold sister chromatids together. Overexpression of a nondegradable *PTTG* disrupts sister chromatid separation (Zou *et al.* [1999]) and overexpression of *PTTG* causes apoptosis and inhibits mitosis (Yu, R. *et al.* [2000]). The securin function of *PTTG* suggests that *PTTG* may also be expressed in normal proliferating cells. In adult animals and humans, *PTTG* mRNA is most abundant in testis (Zhang, X. *et al.* [1999a]); Wang, Z. *et al.* [2000]), an organ containing rapidly proliferating gametes.

A *PTTG* gene family contains at least three genes that share a high degree of sequence homology, including human *PTTG1*, located on chromosome 5q33. (Prezant, T.R., *et al.*, *An intronless homolog of human proto-oncogene hPTTG is expressed in pituitary tumors: evidence for hPTTG family*, J. Clin. Endocrinol. Metab. 84:1149-52 [1999]). Murine *PTTG* shares 66% nucleotide base sequence homology with human *PTTG1* and also exhibits transforming ability. (Wang, Z. and Melmed, S., *Characterization of the murine pituitary tumor transforming gene (PTTG) and its promoter*, Endocrinology [In Press; 2000]). A proline-rich region was identified near the protein C-terminus that is critical for *PTTG1*'s transforming activity. (Zhang, X., *et al.* [1999a]), as demonstrated by the inhibitory effect on in vitro transformation, in vivo tumorigenesis, and transactivation, when point mutations were introduced into the proline-rich region. Proline-rich domains may function as SH3 binding sites to mediate signal transduction of protein-tyrosine kinase. (Pawson, T., *Protein modules and signaling networks*, Nature 373:573-580 [1995]; Kuriyan, J., and Cowburn, D., *Modular peptide recognition domains in eukaryotic signaling*, Annu. Rev. Biophys. Biomol. Struct. 26:259-288 [1997]).

Breast and ovarian cancers are a model of hormone dependent malignancy. Estrogens and progesterone, acting via specific nuclear receptors, are necessary for normal development of mammary gland and ovarian tissue and their differentiated function. In addition to classical estrogenic ligand-estrogen receptor (ER) interactions, and subsequent ER binding to estrogen-response elements to regulate gene transcription, it is now apparent that transcriptional modulation can be mediated through the membranal ER. (Levin E.R., *Cellular functions of the*

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plasma membrane estrogen receptor, TEM 10:374-77 [1999]). This action requires modification of cytosolic signal transduction pathways such as extracellular-signal-regulated kinase/mitogen-activated protein kinase pathways (ERK/MAPK).

5 In breast and ovarian cancers, the molecular mechanisms through which these signal transduction effects are mediated are not well defined, although c-myc and cyclin D1 have been identified as major downstream targets of estrogen and progestin-stimulated cell cycle progression. In addition to regulating cyclin abundance, recruitment of specific CDK inhibitors, such as p21 is impaired by estrogen, and additional, as yet undefined estrogen-regulated components are likely to be regulators of mammary epithelial cell proliferation and differentiation.

10 (Sutherland, R.L., *et al.*, *Estrogen and progestin regulate cell cycle progression*, J. Mammary Gland Biol. Neoplasia 3:63-72 [1998]).

Several studies have described the involvement of SP1 and half-site EREs in conferring estrogen-responsiveness of several genes, including creatine kinase B, c-myc, the retinoic acid receptor α , heat shock protein 27. (Wu-Peng X. *et al.*, *Delineation of sites mediating estrogen*

15 *regulation of the rat creatine kinase B gene*, Mol. Endocrinol. 6:231-240 [1992]; Dubik, D. and Shiu, R., *Mechanism of estrogen activation of c-myc oncogene expression*, Oncogene 7:1587-1594 [1992]). This cooperative interaction of a half-site ERE and an SP1 site has recently been described for the progesterone receptor (Petx, L. and Nardulli, A.M., *Sp1 binding*

20 *sites and an estrogen response element half-site are involved in regulation of the human progesterone receptor A promoter*, Mol. Endocrinol. 14:972-85 [2000]). In the context of complex promoters, EREs are generally found in multiple copies or encased among binding motifs for other transcription factors (Porter, W. *et al.*, *Functional synergy between the transcription*

25 *factor Sp1 and the estrogen receptor*, Mol. Endo. 11:1569-80 [1997]). It has been demonstrated that the SP1 sites on the murine and human PTTG-promoter are crucial for its transactivation activity, and mutational disruption of the SP1 element or competition with a known SP1 oligo

resulted in up to 90% loss of PTTG-promoter activity. (Wang, Z. and Melmed, S., *SP1 activates the pituitary tumor transforming gene (PTTG) promoter during cellular transformation* J Biol Chem [2000]; Kakar, S.S., *Molecular cloning, genomic organization, and identification of the promoter for the human pituitary tumor transforming gene (PTTG)*, Gene 240: 317-324 [1999]).

30 In many solid tumors, tumor vascularity may inversely correlate with prognosis, and both

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